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Mechanisms of Synaptic Alterations in a Neuroinflammation Model of Autism

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14. ABSTRACT

Here we investigated how Maternal Immune Activation (MIA), a risk factor for autism spectrum disorders (ASD) affects the development of synapses. The central hypothesis is that altered expression of cytokines and chemokines in the brain results in impaired synaptic development and altered behavior. We employed the MIA mouse model and so far found that in offspring there is a reduction in the number of synaptic structures in vivo as well as reduced motility. Behavioral impairments relevant to ASD are also reported.

15. SUBJECT TERMS

Nothing listed

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1. Introduction:

Under the aegis of this award we have been investigating how maternal immune activation (MIA), a risk factor for autism spectrum disorder (ASD) affects the development of synapses. The central hypothesis is that altered expression of cytokines and chemokines in the brain results in impaired synaptic development and altered behavior. To test this hypothesis, we employed the MIA mouse model using the viral mimic poly (I:C) to determine how synaptic structure, dynamics and function are affected in the offspring mice. The objective of this proposal is to identify the structural and molecular synaptic alterations induced by maternal activation that could serve in elucidating key mechanisms associated with the pathobiology of ASD. The ability of an anti-inflammatory drug to reverse synaptic and behavioral impairments is also evaluated.

2. Keywords

Autism
Inflammation
Maternal
Cytokines
Synapse
Dendritic Spines
Behavior

3. Overall Project Summary

The major goals of the project:

Task 1: Determine how synapses are altered in offspring challenged by MIA. (Months 1-18)

Task 2: Determine how synapses develop in presence of differential levels of cytokines and chemokines. (Months 6-24)

Task 3: Determine if synaptic and behavioral impairments observed in MIA offspring can be ameliorated by AV411 (Ibudilast), an anti-neuroinflammatory drug. (Months 24-36)

Summary of Results:

Task 1: Determine how synapses are altered in offspring challenged by MIA. (Months 1-18)

1a. Determine if exposure to MIA result in changes in dendritic spine morphology? (1-6)

We measured dendritic spine density and morphology of MIA offspring in the cerebellum and the cortex in mice that express florescent protein in these brain regions. We found that there is a reduction in spine density in the somatosensory cortex of adolescent mice and that this deficits persists into adulthood (Figure 1) mice. We classified spines into categories and found that there is a reduction in density of all spine categories.

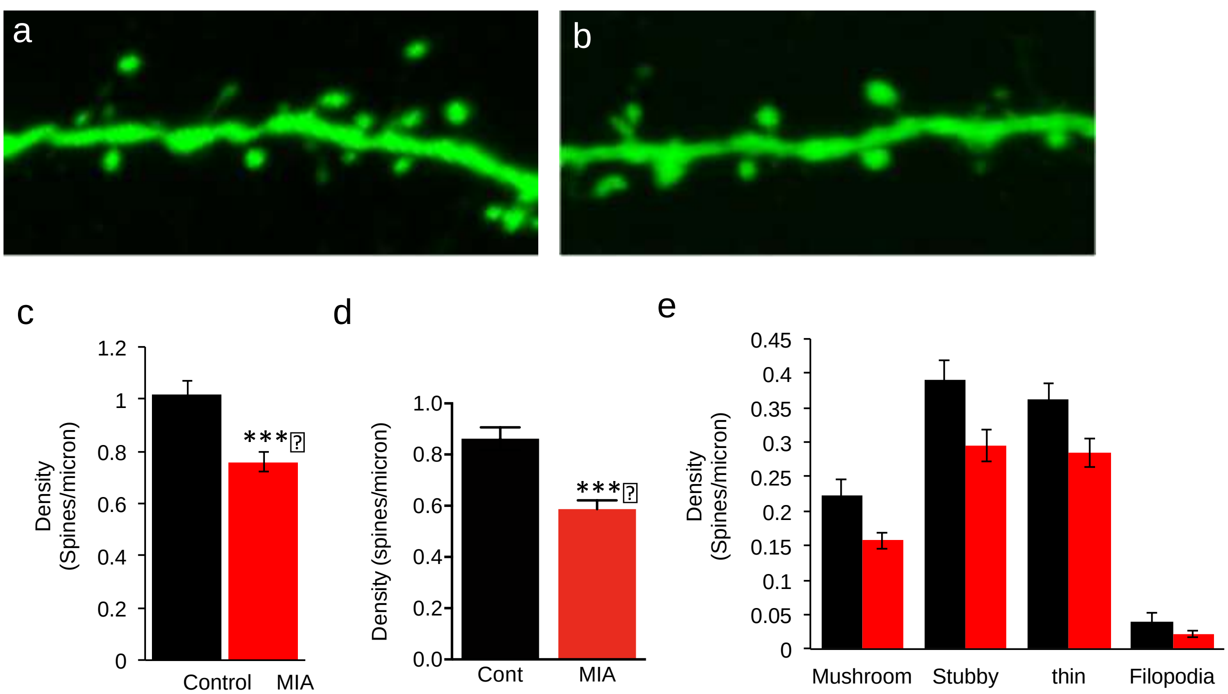


Figure 1: Reduced cortical dendritic spine density in young MIA offspring. Confocal images of layer 5 pyramidal neuron apical tuft dendrites from P17 control **(a)** and MIA **(b)** YFP-H mice. **c.** MIA induces a reduction in total dendritic spine density in P17 offspring. **d.** Spine reduction persists.in 3 month old MIA offspring. **e.** MIA results in a reduction in density of all dendritic spine categories.

Although reduction in number of synapses has been recently shown in dissociated neurons from MIA offspring in culture, our results are the first to demonstrate a reduction in vivo.

The analysis of dendritic spines on the cerebellar Purkinje neurons is ongoing.

1b. Determine if exposure to MIA result in changes in dendritic spine dynamic by using the Motility Index

We have performed in vivo imaging of dendritic spines of MIA and control offspring through a cranial window. We have confirmed that reduced spine density is observed when the intact brain is imaged. Importantly we discovered that dendritic spines in MIA offspring are much less dynamic (Fig. 2). Dendritic spine dynamics is thought to be essential for the proper formation of synaptic contacts and neuronal circuits.

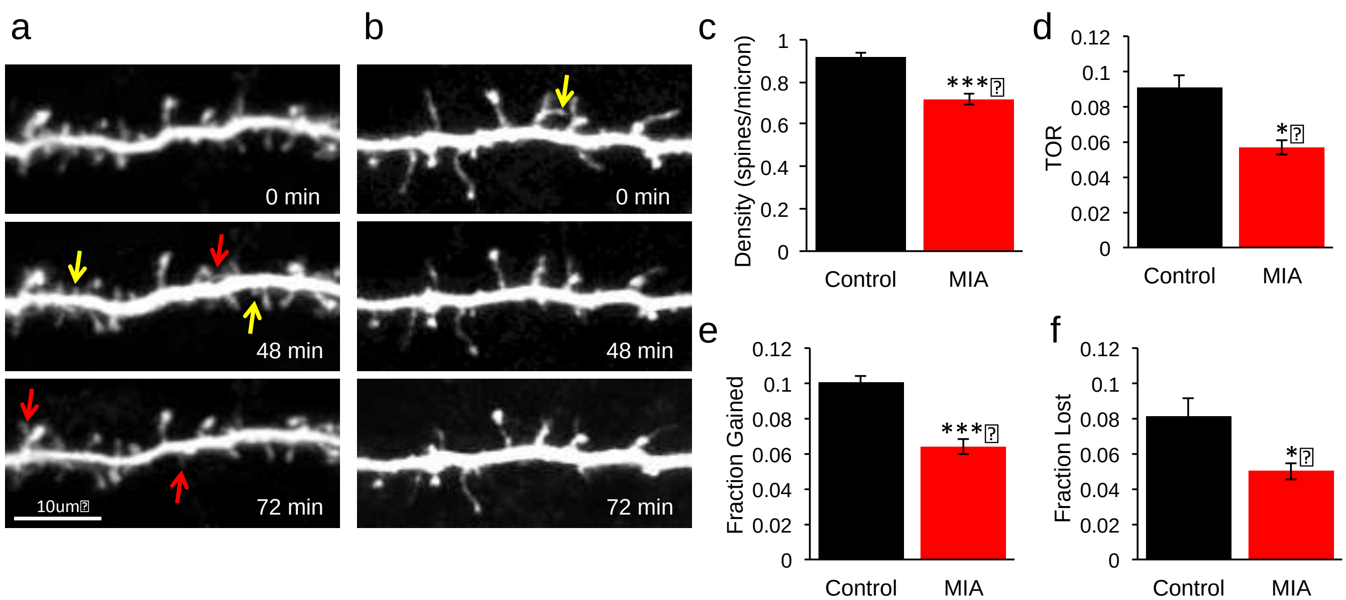
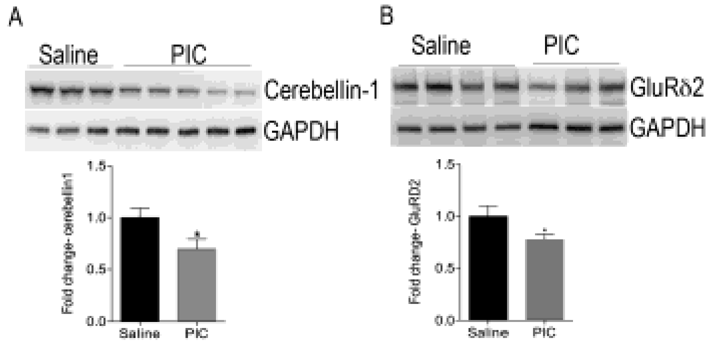


Figure 2: Impaired dynamics of dendritic spines in MIA offspring. Two-photon imaging of cortical neurons from P17 YFP-H mice through a thinned skull window in control **(a)** and MIA **(b)** offspring. Images were collected every 12 minutes for a period of 1.5 hrs. Note the reduced density of dendritic protrusions in the MIA offspring. Dendritic spine formation (red arrows) and elimination (yellow arrows) is prevalent in control but not MIA offspring. **c-f.** Quantification of spine parameters imaged in vivo in 5 mice for each condition. Spine density is reduced in MIA offspring (control: 0.92±0.02 spines/□m; MIA: 0.72±0.02 spines/□m, P=0.0001). Turnover rate (TOR) is reduced in MIA offspring (control: 0.091±0.01; MIA 0.057±0.004, P=0.013) due to reduction in both fraction of spines gained (control: 0.1±0.003 MIA: 0.064±0.004, P<0.0001) and reduced fraction of spines lost (control: 0.08±0.01; MIA: 0.05±0.005, P=0.028)

1c. Determine if synaptic protein expression signatures altered in the MIA offspring by employing quantitative mass spectrometry based proteomics and array tomography.

We have performed preliminary iTRAQ experiments on the synaptosomal proteome isolated from P14 cerebella of MIA. We performed western blot analyses to confirm some of the hits found through the high throughput analysis. We were not able to confirm the decrease in doublecortin. We did confirm a reduction in cerebelin1, an important synaptic organizer in the cerebellum of MIA offspring (Fig. 3). Moreover we have also determined that there is a reduction in GluRδ2 a receptor for cerebelin 1.



re 3: Reduced levels of cerebelin 1 and ƷluRδ2 in the cerebellum of MIA (PIC) ıffspring.
Vestern blot analysis of synaptosomes isolated rom postnatal day 14 cerebellum demonstrates reduction in the PIC (MIA) offspring. **B.** A imilar result is observed in western blots for the eceptor GluRd2.

This is the first report of reduction in levels of a synaptic organizer in the MIA offspring and might be related to the reduction in number of synaptic structures observed.

In ongoing experiments we are determining if presynaptic inputs (visualized with excitatory and inhibitory synaptic proteins, VGluT1 and GAD-6) are altered in MIA offspring.

Task 3: Determine if synaptic and behavioral impairments observed in MIA offspring can be ameliorated by AV411 (Ibudilast), an anti-neuroinflammatory drug. (Months 24-36)
In preparation for this aim we have determined that MIA offspring exhibit behavioral impairments. We have found that PCP2 MIA offspring have reduced Ultrasonic Vocalization, increased marble burying (assay for repetitive behavior) and decreased social interaction. These behaviors are thought to be relevant to the core behavioral deficits found in individuals with ASD.

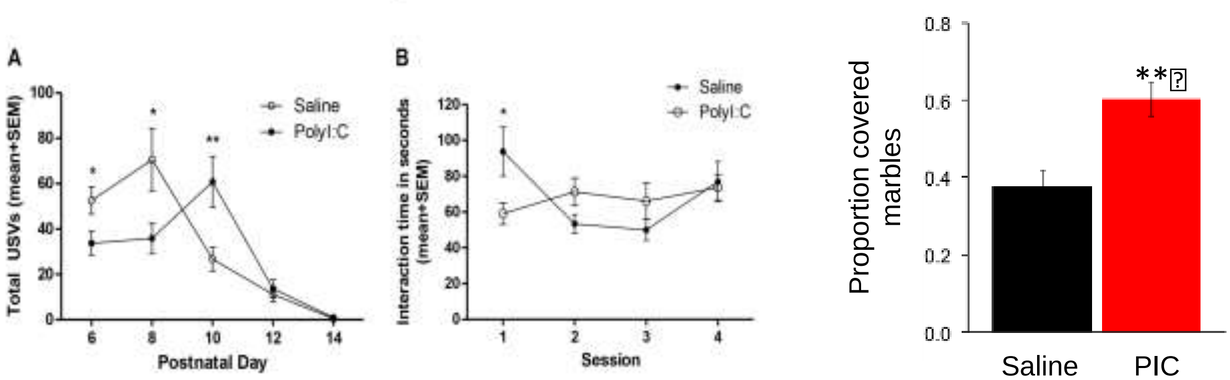


Figure 4: Impaired ASD relevant behaviors in MIA offspring. A. MIA offspring (PIC) had emitted fewer USVs during postnatal days 6 and 8. **B.** MIA offspring (PolyI:C) spent less time with an unfamiliar (sessions 1 &4) and did not habituate as much as the control (saline) offspring. **C.** MIA offspring buried more marbles than control mice.

4. Key Research Accomplishments

- We have determined that dendritic spine density, sites of excitatory synaptic input, is reduced in both adolescent and adult MIA mice.
- We have determined that dendritic spine dynamics is reduced in MIA mice.

- We have determined the level of proteins with defined roles in synaptic organization are reduced in MIA mice.

5. Conclusions

Our results so far will have high impact on the field as this is the first analysis of synaptic deficits in vivo and spine dynamics have never before been examined in the MIA offspring. Moreover we show that spine deficits are maintained into adulthood, potentially explaining the behavioral deficits observed in the adult MIA mice.

6. Publications, abstracts and presentations:

These results have been presented at the annual Society for Neuroscience meeting:

Pendyala, G., Ragunathan, P., Jung, Y., Suresh, A., Meays, B., Spartz, E., Dunaevsky, A. (2013) *Impaired synaptic development with maternal immune activation. Soc. Neurosci. Abstr* 28.06

Our results were also highlighted by SFARI;
http://sfari.org/news-and-opinion/conference-news/2013/society-for-neuroscience-2013/maternal-infection-may-alter-neuronal-signals-connections

7. Inventions, Patents and Licenses

Nothing to report

8. Reportable Outcomes

Nothing to report

9. Other Achievements:

Nothing to report

10. References

NA

11. Appendices

NA